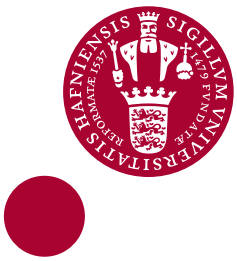

Fast and efficient single step construction of replacement vectors by USER Friendly cloning, for targeted gene replacement in fungi

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Title:

Fast and efficient single step construction of replacement vectors by USER Friendly cloning, for targeted gene replacement in fungi

Abstract:

Functional genetics in filamentous fungi have always been dependent on the isolation or construction of mutant strains. The genome sequencing of over 40 fungi genomes has increased the need for faster and more efficient methods to construct targeted replacement and overexpression mutants. To accommodate this we have developed a new vector system that allows single step construction of vectors for targeted gene replacement, thereby cutting vector construction time from ten to only three days and removing half of the required work load. The vector system is dependent on the Uracil-Specific Excision Reagent cloning technology (USER Friendly™), which in its commercial version offers high efficient directional cloning of a single PCR amplicon. However, our research shows that USER friendly™ cloning technology also can be used for the simultaneous directional cloning of several PCR amplicons and vector fragments, with a cloning efficiency of 85 %, thus allowing single-step construction of replacement vectors. In addition to the increased speed and reduced workload, the single-step construction strategy also offers greater freedom of operation with respect to the placing of the homologous recombination flanks, as it is independent of restriction enzymes.
The new vector system includes vectors for targeted gene replacement (pRF-HU2), promoter exchange (pRF-HU2E), ectopic overexpression (pRF-HUE) and general purpose cloning (pRF-HU). All are compatible with both protoplast and *Agrobacterium tumefaciens* mediated transformation technologies. The system has been used to analyse putative polyketide gene clusters in *Fusarium graminearum*.